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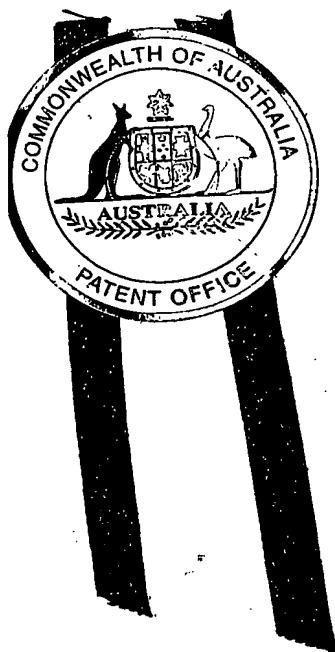
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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND  
SALES hereby certify that annexed is a true copy of the Provisional specification  
in connection with Application No. 2003902452 for a patent by THE  
UNIVERSITY OF QUEENSLAND as filed on 20 May 2003.



WITNESS my hand this  
Fourth day of November 2003

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AUSTRALIA  
Patents Act 1990

**PROVISIONAL SPECIFICATION**

**Applicant:**

THE UNIVERSITY OF QUEENSLAND

**Invention Title:**

TREATMENT OF INFLAMMATORY BOWEL DISEASE

The invention is described in the following statement:

TREATMENT OF INFLAMMATORY BOWEL DISEASE

FIELD OF THE INVENTION

This invention relates to the treatment of  
5 inflammatory bowel disease with novel cyclic peptidic and  
peptidomimetic compounds which have the ability to  
modulate the activity of G protein-coupled receptors. The  
compounds preferably act as antagonists of the C5a  
receptor, and are active against C5a receptors on  
10 polymorphonuclear leukocytes and macrophages.

BACKGROUND OF THE INVENTION

All references, including any patents or patent  
applications, cited in this specification are hereby  
15 incorporated by reference. No admission is made that any  
reference constitutes prior art. The discussion of the  
references states what their authors assert, and the  
applicants reserve the right to challenge the accuracy and  
pertinency of the cited documents. It will be clearly  
20 understood that, although a number of prior art  
publications are referred to herein, this reference does  
not constitute an admission that any of these documents  
forms part of the common general knowledge in the art, in  
Australia or in any other country.

25 G protein-coupled receptors are prevalent  
throughout the human body, comprising approximately 60% of  
known cellular receptor types, and mediate signal  
transduction across the cell membrane for a very wide  
range of endogenous ligands. They participate in a  
30 diverse array of physiological and pathophysiological  
processes, including, but not limited to those associated  
with cardiovascular, central and peripheral nervous  
system, reproductive, metabolic, digestive, immunological,  
inflammatory, and growth disorders, as well as other cell-  
35 regulatory and proliferative disorders. Agents which  
selectively modulate functions of G protein-coupled  
receptors have important therapeutic applications. These

receptors are becoming increasingly recognised as important drug targets, due to their crucial roles in signal transduction (G protein-coupled Receptors, IBC Biomedical Library Series, 1996).

5           One of the most intensively studied G protein-coupled receptors is the receptor for C5a. C5a is one of the most potent chemotactic agents known, and recruits neutrophils and macrophages to sites of injury, alters their morphology; induces degranulation; increases calcium  
10 mobilisation, vascular permeability (oedema) and neutrophil adhesiveness; contracts smooth muscle; stimulates release of inflammatory mediators, including histamine, TNF- $\alpha$ , IL-1, IL-6, IL-8, prostaglandins, and leukotrienes, and of lysosomal enzymes; promotes formation  
15 of oxygen radicals; and enhances antibody production (Gerard and Gerard, 1994).

          Agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these  
20 reasons, molecules which prevent C5a from binding to its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Because such compounds act upstream from the various inflammatory mediators referred to above, and inhibit the formation of  
25 many of these compounds, they may have a more powerful effect in alleviating or preventing inflammatory symptoms.

          In our previous applications No.PCT/AU98/00490, we described the three-dimensional structure of some analogues of the C-terminus of human C5a, and used this  
30 information to design novel compounds which bind to the human C5a receptor (C5aR), behaving as either agonists or antagonists of C5a. It had previously been thought that a putative antagonist might require both a C-terminal arginine and a C-terminal carboxylate for receptor binding  
35 and antagonist activity (Kontetis et al, 1994). We showed that in fact a terminal carboxylate group is not generally required either for high affinity binding to

C5aR or for antagonist activity. Instead we found that a hitherto unrecognised structural feature, a turn conformation, was the key recognition feature for high affinity binding to the human C5a receptor on neutrophils.

5 As described in our Australian provisional application No. PR8334, filed on 17<sup>th</sup> October 2001, we used these findings to design constrained structural templates which enable hydrophobic groups to be assembled into a hydrophobic array for interaction with a C5a receptor.

10 The entire disclosures of these specifications are incorporated herein by this reference.

Inflammatory bowel disease (IBD) is a group of serious, chronic relapsing inflammatory diseases affecting both the small and large intestine, which remains

15 relatively resistant to current treatments. IBD is characterized by spontaneously occurring, chronic relapsing inflammation of unknown origin, in which current treatment options are inadequate (reviewed by van Deventer, 2002). Despite extensive research into the

20 disease in both humans and experimental animals, the precise mechanisms of pathology remain to be elucidated. A host of immune and inflammatory mediators are thought to be involved, including biogenic amines, kinins, arachidonic acid metabolites, free radicals, nitric oxide,

25 various proinflammatory cytokines, and complement proteins (Nielsen et al, 1996; Papadakis et al, 2000).

Current therapies for IBD target one or more of these inflammatory mediators. Recent advances in drug development for IBD have involved the use of monoclonal

30 antibodies to inhibit pro-inflammatory cytokines, such as interleukins, interferons and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) (Sandborn et al, 2002). In particular, the anti-TNF- $\alpha$  antibodies CDP571 and infliximab have been used clinically to treat Crohn's disease with some success.

35 However, these new protein therapies suffer from major drawbacks, such as the costs of production, instability, poor bioavailability, limited routes of administration,

and immunogenicity.

Several major forms of IBD are known, and Crohn's disease (regional bowel disease) and ulcerative colitis are the most common of these disorders. Because of the nature of their pathology, there are a number of autoimmune and immune-mediated diseases of the small and large bowel which are likely to benefit from treatment with these C5a antagonists. These include lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis. These conditions have been diagnosed in humans and in a number of animal species.

In 1999, approximately 1.7 million people in the United States alone were diagnosed with this debilitating disease. Satisfactory treatment of IBD is an unmet medical need, as existing therapeutic agents have not been successful in curtailing the disease and avoiding the need for surgery. Up to 40% of all ulcerative colitis patients undergo surgery, which typically includes either the removal of part of the large intestine or a full colectomy. While surgery is not curative for Crohn's disease, 75% of all patients will undergo at least one surgery in their lifetime, and up to 90% of these patients require additional surgeries. A therapeutic agent which can successfully treat inflammatory bowel disease can enormously improve a patient's quality of life, while potentially saving the healthcare system millions of dollars in costs associated with invasive surgical procedures.

In these conditions, chronic inflammation, caused by a number of inflammatory mediators, has been implicated in pathogenesis. The complement system has been acknowledged as one of these inflammatory mediators, since increased levels of complement products are found in the colons of patients with IBD (Nielsen et al. 1996). Ulcerative colitis, also known as idiopathic colitis, is characterized by inflammation of the colon and rectum,

which become inflamed and ulcerated; its cause is unclear, although antibodies to colonic epithelium and *E. coli* strain 0119 B14 are often present. Its severity varies, and the patients suffer frequent relapses. Crohn's  
5 disease, also called regional enteritis or regional ileitis, is characterized by inflammation, thickening and ulceration of the bowel wall, usually in the terminal part of the ileum, with oedematous mucosa or thickened soft  
10 tissue, mesenteric infiltration, thickened bowel wall, and often inflammatory masses, abscesses, or distended fluid-filled loops. Complications include fistulae, intramural sinus tracts, abscesses, perforations, toxic megacolon, obstruction of the bowel, or hydronephrosis, and there is an increased risk of adenocarcinoma in the ileum or colon.

15 In both these conditions extensive bed rest is often required, and in severe case the affected part of the bowel may be surgically removed, necessitating the use of an ostomy bag. The only therapeutic agents which are available are corticosteroids such as prednisolone,  
20 monoclonal antibodies directed against tumour necrosis factor, such as Remicade (infliximab), immunosuppressive agents such as methotrexate, azathioprine, cyclosporine, tacrolimus and mycophenolate mofetil, or other anti-inflammatory agents such as sulphasalazine. These agents,  
25 especially the steroids, may be of limited effectiveness, and may have serious side effects. A variety of other agents, including corticosteroid derivatives such as budesonide antagonists and agonists of cytokines such as interleukin-10 and interleukin 11, and a nicotinic  
30 receptor agonist, are in various stages of clinical trial. However, there is a great need in the art for effective, non-toxic agents which do not require administration by injection, and which can be produced at reasonable cost. To our knowledge none of these approved or experimental  
35 agents, and in particular no small molecule agent, targets the C5a receptor.

Due to the current lack of knowledge of

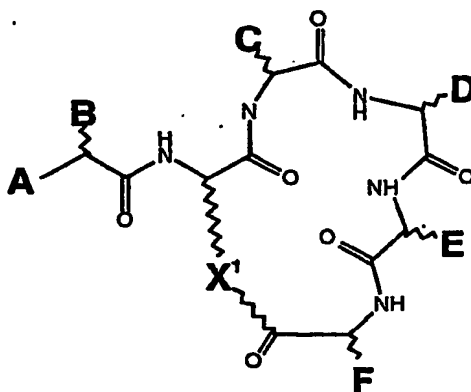
complement involvement in IBD, we tested the possible inhibitory effects of specific complement inhibitors in an animal model of colitis. We now show for the first time that a specific inhibitor of the C5a receptor is able to ameliorate signs of damage in trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats. This model has been used extensively to investigate the pathogenesis of IBD (Morris et al, 1989). We found strong protective effects for both the C3a and C5a antagonists, suggesting a large and previously undefined role for complement as a mediator of IBD.

This is the first reported case of an inhibitor of the complement system being used to modulate pathology in a model of IBD.

#### SUMMARY OF THE INVENTION

According to a first aspect, the invention provides a method of treatment of inflammatory bowel disease (IBD), comprising the step of administering an effective amount of an inhibitor of a G protein-coupled receptor to a subject in need of such treatment.

- Preferably the a inhibitor is a compound which
- is an antagonist of a G protein-coupled receptor,
  - has substantially no agonist activity, and
  - is a cyclic peptide or peptidomimetic compound of formula I





where A is H, alkyl, aryl, NH<sub>2</sub>, NH-alkyl, N(alkyl)<sub>2</sub>, NH-aryl, NH-acyl, NH-benzoyl, NHSO<sub>3</sub>, NHSO<sub>2</sub>-alkyl, NHSO<sub>2</sub>-aryl, OH, O-alkyl, or O-aryl;

5           B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid such as L-phenylalanine or L-phenylglycine, but is not the side chain of glycine, D-phenylalanine, L-homophenylalanine, L-tryptophan, L-homotryptophan, L-tyrosine, or L-homotyrosine;

10           C is a small substituent, such as the side chain of a D-, L- or homo-amino acid such as glycine, alanine, leucine, valine, proline, hydroxyproline, or thioproline, but is preferably not a bulky substituent such as isoleucine, phenylalanine, or cyclohexylalanine;

15           D is the side chain of a neutral D-amino acid such as D-Leucine, D-homoleucine, D-cyclohexylalanine, D-homocyclohexylalanine, D-valine, D-norleucine, D-homo-norleucine, D-phenylalanine, D-tetrahydroisoquinoline, D-glutamine, D-glutamate, or D-tyrosine, but is preferably not a small substituent such as the side chain of glycine or D-alanine, a bulky planar side chain such as D-tryptophan, or a bulky charged side chain such as D-arginine or D-Lysine;

25           E is a bulky substituent, such as the side chain of an amino acid selected from the group consisting of L-phenylalanine, L-tryptophan and L-homotryptophan, or is L-1-naphthyl or L-3-benzothienyl alanine, but is not the side chain of D-tryptophan, L-N-methyltryptophan, L-homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline, L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or L-histidine;

30           F is the side chain of L-arginine, L-homoarginine, L-citrulline, or L-canavanine, or a bioisostere thereof, ie. a side chain in which the terminal guanidine or urea group is retained, but the carbon backbone is replaced by a group which has different

structure but is such that the side chain as a whole reacts with the target protein in the same way as the parent group; and

5 X is  $-(CH_2)_nNH-$  or  $(CH_2)_nS-$ , where n is an integer of from 1 to 4, preferably 2 or 3;  $-(CH_2)_2O-$ ;  $-(CH_2)_3O-$ ;  $-(CH_2)_3-$ ;  $-(CH_2)_4-$ ;  $-CH_2COCHRNH-$ ; or  $-CH_2-CHCOCHRNH-$ , where R is the side chain of any common or uncommon amino acid.

10 In C, both the *cis* and *trans* forms of hydroxyproline and thioproline may be used.

Preferably A is an acetamide group, an aminomethyl group, or a substituted or unsubstituted sulphonamide group.

15 Preferably where A is a substituted sulphonamide, the substituent is an alkyl chain of 1 to 6, preferably 1 to 4 carbon atoms, or a phenyl or toluyl group.

In a particularly preferred embodiment, the compound has antagonist activity against C5aR, and has no C5a agonist activity.

20 The compound is preferably an antagonist of C5a receptors on human and mammalian cells including, but not limited to, human polymorphonuclear leukocytes and human macrophages. The compound preferably binds potently and selectively to C5a receptors, and more preferably has  
25 potent antagonist activity at sub-micromolar concentrations. Even more preferably the compound has a receptor affinity  $IC_{50} < 25 \mu M$ , and an antagonist potency  $IC_{50} < 1 \mu M$ .

30 Most preferably the compound is compound 1, compound 33, compound 60 or compound 45 described in Provisional application no. PR8334.

The inhibitor may be used in conjunction with one or more other agents for the treatment of IBD, including but not limited to corticosteroids such as prednisolone  
35 and budesonide, other immunosuppressive agents such as infliximab (Remicade; Johnson & Johnson), methotrexate or azathioprine, anti-inflammatory agents such as

sulphasalazine, Colazal (balsalazide), and the like, and probiotics.

The compositions of the invention may be formulated for oral, parenteral, inhalational, intranasal, 5 rectal or transdermal use, but oral or topical formulations are preferred. It is expected that most if not all compounds of the invention will be stable in the presence of metabolic enzymes, such as those of the gut, blood, lung or intracellular enzymes. Such stability can 10 readily be tested by routine methods known to those skilled in the art.

Suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known textbooks such as 15 Remington: The Science and Practice of Pharmacy, Vol. II, 2000 (20<sup>th</sup> edition), A.R. Gennaro (ed), Williams & Wilkins, Pennsylvania.

While the invention is not in any way restricted to the treatment of any particular animal or species, it 20 is particularly contemplated that the method of the invention will be useful in medical treatment of humans, and will also be useful in veterinary treatment, particularly of companion animals such as cats and dogs, livestock such as cattle, horses and sheep, and zoo 25 animals, including non-human primates, large bovids, felids, ungulates and canids.

The compound may be administered at any suitable dose and by any suitable route. Oral, transdermal or intranasal administration is preferred, because of the 30 greater convenience and acceptability of these routes. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of health of the individual treatment. This will be at the discretion of the attending physician 35 or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the effect on mortality rate over  
5 8 days. C5aA = C5a antagonist; Pred = prednisolone.

Figure 2 shows the effect on food consumption.  
The amount of food consumed was measured either after 24 h  
[A] or at 8 days [B]. Data represent the mean  $\pm$  SEM (n =  
4-12). \*P < 0.05 drug-treated rats vs. colitis control.  
10 C5aA = C5a antagonist; C3aA = C3a antagonist; Pred =  
prednisolone.

Figure 3 shows the effect on body weight. The  
change in body weight following TNBS administration was  
measured after either [A] 24 h or [B] 8 days. Data  
15 represent the mean  $\pm$  SEM (n = 4-12). \*P < 0.05 drug-  
treated rats vs. colitis control. C5aA = C5a antagonist;  
C3aA = C3a antagonist; Pred = prednisolone.

Figure 4 summarizes the clinical macroscopic  
scores. The colons were scored for macroscopic damage on  
20 a scale of 0-13 at either [A] 24 h or [B] 8 days. Data  
represent the mean  $\pm$  SEM (n = 4-12). \*P < 0.05 drug-  
treated rats vs. colitis control. C5aA = C5a antagonist;  
C3aA = C3a antagonist; Pred = prednisolone.

Figure 5 shows the effect on colon  
25 myeloperoxidase (MPO) levels. Levels of MPO in rat's  
colons were measured at either [A] 24 h or [B] 8 days.  
Data represent the mean  $\pm$  SEM (n = 4-12). \*P < 0.05 drug-  
treated rats vs. colitis control. C5aA = C5a antagonist;  
C3aA = C3a antagonist; Pred = prednisolone.

30 Figure 6 shows the effect on colon oedema levels  
in 24 h and 8 day studies. [A] 24 h; [B] 8 days. Data  
represent the mean  $\pm$  SEM (n = 4-12). \*P < 0.05 drug-  
treated rats vs. colitis control. C5aA = C5a antagonist;  
C3aA = C3a antagonist; Pred = prednisolone.

35 Figure 7 shows the effect on serum and colonic  
TNF- $\alpha$  levels after 8 days. Data represent the mean  $\pm$  SEM  
(n = 4-12).

[A] serum; [B] colonic tissue.

\*P < 0.05 drug-treated rats vs. colitis control. C5aA = C5a antagonist; C3aA = C3a antagonist; Pred = prednisolone.

5                   Figure 8 shows sections of colons from rats in the 8 day study, stained with haematoxylin and eosin (x 40 magnification). Images are typical, and representative of each study group.

10           [A] saline injected, sham-operated rat with intact epithelial and mucosal layer (ML);

[B] TNBS drug-free colitis control rat with extensive damage including inflammatory cell (IC) infiltration, oedema (E), and the complete destruction of ML architecture (indicated by arrows);

15           [C] TNBS C5a antagonist (10 mg/kg/day, oral) post-treated rat with IC infiltration and E, but with intact ML; and

[D] TNBS prednisolone (1 mg/kg/day, SC) post-treated rat with IC infiltration, E, extensive haemorrhage (H) and erosion of ML (indicated by arrows).

20

#### DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described by way of reference only to the following general methods and experimental examples.

25           For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

30           As used herein, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an enzyme" includes a plurality of such enzymes, and a reference to "an amino acid" is a reference to one or more amino acids. Unless defined otherwise, all technical and  
35           scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and

methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

Abbreviations used herein are as follows:

5	Cit	citrulline
	dCha	D-cyclohexylamine
	DPhe	D-phenylalanine
	IBD	inflammatory bowel disease
	ip	intraperitoneal
10	iv	intravenous
	LPS	lipopolysaccharide
	MPO	myeloperoxidase
	PMN	polymorphonuclear granulocyte
	PMSF	phenylmethylsulfonyl fluoride
15	sc	subcutaneous
	TNBS	trinitrobenzenesulphonic acid
	TNF- $\alpha$	tumour necrosis factor- $\alpha$

Throughout the specification conventional single-  
20 letter and three-letter codes are used to represent amino acids.

For the purposes of this specification, the term "alkyl" is to be taken to mean a straight, branched, or cyclic, substituted or unsubstituted alkyl chain of 1 to  
25 6, preferably 1 to 4 carbons. Most preferably the alkyl group is a methyl group. The term "acyl" is to be taken to mean a substituted or unsubstituted acyl of 1 to 6, preferably 1 to 4 carbon atoms. Most preferably the acyl group is acetyl. The term "aryl" is to be understood to  
30 mean a substituted or unsubstituted homocyclic or heterocyclic aryl group, in which the ring preferably has 5 or 6 members.

A "common" amino acid is a L-amino acid selected from the group consisting of glycine, leucine, isoleucine,  
35 valine, alanine, phenylalanine, tyrosine, tryptophan, aspartate, asparagine, glutamate, glutamine, cysteine, methionine, arginine, lysine, proline, serine, threonine

and histidine.

An "uncommon" amino acid includes, but is not restricted to, D-amino acids, homo-amino acids, N-alkyl amino acids, dehydroamino acids, aromatic amino acids  
5 other than phenylalanine, tyrosine and tryptophan, ortho-, meta- or para-aminobenzoic acid, ornithine, citrulline, canavanine, norleucine,  $\gamma$ -glutamic acid, aminobutyric acid, L-fluorenylalanine, L-3-benzothienylalanine, and  $\alpha,\alpha$ -disubstituted amino acids.

10 Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or  
15 sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a disease.

"Treating" as used herein covers any treatment of, or prevention of disease in a vertebrate, a mammal, particularly a human, and includes: preventing the disease  
20 from occurring in a subject who may be predisposed to the disease, but has not yet been diagnosed as having it; inhibiting the disease, i.e., arresting its development; or relieving or ameliorating the effects of the disease, i.e., cause regression of the effects of the disease.

25 The invention includes the use of various pharmaceutical compositions useful for ameliorating disease. The pharmaceutical compositions according to one embodiment of the invention are prepared by bringing a compound of formula I, analogue, derivatives or salts  
30 thereof and one or more pharmaceutically-active agents or combinations of compound of formula I and one or more pharmaceutically-active agents into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries.

35 Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch,

vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient  
5 replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for  
10 instance, in Remington's Pharmaceutical Sciences, 20th ed. Williams & Wilkins (2000) and The British National Formulary 43rd ed. (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2002; <http://bnf.rhn.net>), the contents of which are hereby  
15 incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed., 1985).

20 The pharmaceutical compositions are preferably prepared and administered in dosage units. Solid dosage units include tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of  
25 the disorder, age and body weight of the subject, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of  
30 an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention may be administered locally or systemically in a  
35 therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject.



Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of the cytotoxic side effects. Various considerations are described, eg. in Langer, Science, 249: 1527, (1990). Formulations for oral use may be in the form of hard gelatin capsules, in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules, in which the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients may be suspending agents such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, which may be

- (a) a naturally occurring phosphatide such as lecithin;
- (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate;
- (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol;
- (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or
- (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according

to known methods using suitable dispersing or wetting agents and suspending agents such as those mentioned above. The sterile injectable preparation may also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents which may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

Compounds of formula I may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

Dosage levels of the compound of formula I of the present invention will usually be of the order of about 0.5mg to about 20mg per kilogram body weight, with a preferred dosage range between about 0.5mg to about 10mg per kilogram body weight per day (from about 0.5g to about 3g per patient per day). The amount of active ingredient which may be combined with the carrier materials to produce a single dosage will vary, depending upon the host to be treated and the particular mode of administration. For example, a formulation intended for oral administration to humans may contain about 5mg to 1g of an active compound with an appropriate and convenient amount of carrier material, which may vary from about 5 to 95 percent of the total composition. Dosage unit forms will generally contain between from about 5mg to 500mg of active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

In addition, some of the compounds of the invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

The compounds of the invention may additionally be combined with other therapeutic compounds to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not eliminate the activity of the compound of formula I of this invention.

## **General Methods**

### **Peptide synthesis**

Cyclic peptide compounds of formula I are prepared according to methods described in detail in our earlier applications No. PCT/AU98/00490 and PR8334, the entire disclosures of which are incorporated herein by this reference. While the invention is specifically illustrated with reference to the compound AcF-[OPdChawR] (PMX53), whose corresponding linear peptide is Ac-Phe-Orn-Pro-dCha-Trp-Arg, it will be clearly understood that the invention is not limited to this compound.

Compounds 1-6, 17, 20, 28, 30, 31, 36 and 44 disclosed in International patent application No. PCT/AU98/00490 and compounds 10-12, 14, 15, 25, 33, 35, 40, 45, 48, 52, 58, 60, 66, and 68-70 disclosed for the first time in Australian provisional application No. PR8334 have appreciable antagonist potency ( $IC_{50} < 1 \mu M$ )

against the C5a receptor on human neutrophils. PMX53 and compounds 33, 45 and 60 of PR8334 are most preferred.

We have found that all of the compounds of formula I which have so far been tested have broadly  
5 similar pharmacological activities, although the physicochemical properties, potency, and bioavailability of the individual compounds varies somewhat, depending on the specific substituents.

The general tests described below may be used for  
10 initial screening of candidate inhibitor of G protein-coupled receptors, and especially of C5a receptors.

#### **Drug preparation and formulation**

The human C5a receptor antagonist AcF-[OPdChaWR]  
15 (AcPhe[Orn-Pro-D-Cyclohexylalanine-Trp-Arg]) was synthesized as described above, purified by reversed phase HPLC, and fully characterized by mass spectrometry and proton NMR spectroscopy. The C5a antagonist was prepared in olive oil (10 mg/mL) for oral dosing and in a 30%  
20 polyethylene glycol solution (0.6 mg/mL) for SC dosing. The C3a antagonist SB 290157 ( $\text{Ph}_2\text{CHCH}_2\text{OCH}_2\text{CO-Arg-OH}$ ) has been described in detail previously (Ames et al, 2001). It was synthesized and purified by reversed phase HPLC and characterized by mass spectrometry and NMR spectroscopy.  
25 It was prepared in a 50% propylene glycol solution (30 mg/kg) for IP injections. The glucocorticosteroid, prednisolone (Sigma, U.S.A.) was prepared in a 30% polyethylene glycol solution for SC dosing. The TNF- $\alpha$  inhibitor infliximab (Remicade®) was prepared in sterile  
30 saline (3 mg/mL) as per instructions for IV dosing. No anti-inflammatory effects were observed for propylene glycol or polyethylene glycol in rats with TNBS-induced colitis.

#### **35 Receptor-Binding Assay**

Assays are performed with fresh human PMNs, isolated as previously described (Sanderson et al, 1995),

using a buffer of 50 mM HEPES, 1 mM  $\text{CaCl}_2$ , 5 mM  $\text{MgCl}_2$ , 0.5% bovine serum albumin, 0.1% bacitracin and 100  $\mu\text{M}$  phenylmethylsulfonyl fluoride (PMSF). In assays performed at 4°C, buffer, unlabelled human recombinant C5a (Sigma) or peptide, Hunter/Bolton labelled  $^{125}\text{I}$ -C5a (~ 20 pM) (New England Nuclear, MA) and PMNs ( $0.2 \times 10^6$ ) are added sequentially to a Millipore Multiscreen assay plate (HV 0.45) having a final volume of 200  $\mu\text{L}$ /well. After incubation for 60 min at 4°C, the samples are filtered and the plate washed once with buffer. Filters are dried, punched and counted in an LKB gamma counter. Non-specific binding is assessed by the inclusion of 1mM peptide or 100 nM C5a, which typically results in 10-15% total binding.

Data are analysed using non-linear regression and statistics with Dunnett post-test.

#### **Myeloperoxidase Release Assay for Antagonist Activity**

Cells are isolated as previously described (Sanderson et al, 1995) and incubated with cytochalasin B (5 $\mu\text{g}/\text{mL}$ , 15 min, 37°C). Hank's Balanced Salt solution containing 0.15% gelatin and peptide is added on to a 96 well plate (total volume 100  $\mu\text{L}$ /well), followed by 25  $\mu\text{L}$  cells ( $4 \times 10^6/\text{mL}$ ). To assess the capacity of each peptide to antagonise C5a, cells are incubated for 5 min at 37°C with each peptide, followed by addition of C5a (100 nM) and further incubation for 5 min. Then 50  $\mu\text{L}$  of sodium phosphate (0.1M, pH 6.8) is added to each well, the plate was cooled to room temperature, and 25  $\mu\text{L}$  of a fresh mixture of equal volumes of dimethoxybenzidine (5.7 mg/mL) and  $\text{H}_2\text{O}_2$  (0.51%) is added to each well. The reaction is stopped at 10 min by addition of 2% sodium azide. Absorbances are measured at 450 nm in a Bioscan 450 plate reader, corrected for control values (no peptide), and analysed by non-linear regression.

Example 1:      Effect of PMX53 on inflammatory bowel  
                         disease

5 Due to the current lack of knowledge of complement involvement in IBD, we aimed to test possible inhibitory effects for PMX53 (AcF-[OPdChaWR]) in trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. For comparison, we also examined the efficacy of a C3a antagonist SB 290157 (Ames et, 2001), the corticosteroid prednisolone, and the TNF- $\alpha$  antibody infliximab.

PMX53 (AcF-[OpdChaWR]) was tested for its ability to ameliorate signs of damage in trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats. This model has been used extensively to investigate the pathogenesis of IBD (Morris et al, 1989).

Male Wistar rats (250-300g) were starved for 24 hours prior to being anaesthetised with ketamine (80 mg/kg i.p.) and xylazine (8 mg/kg i.p.). A 1.7 mm outer diameter polyethylene catheter was then inserted intracolonicallly to a distance of 8cm from the anus. 120mg/kg TNBS (50mg/mL solution) along with 250µL ethanol (100%) was then instilled to rats, which remained in the head-down position for 30 min to prevent leakage. Sham-operated rats (rats without colitis) received either saline alone or 250µL ethanol and saline. Rats were then allowed to recover under observation.

At the completion of each experiment, rats were anaesthetized with zolazepam (50 mg/kg, IP) and xylazine (12 mg/kg, IP), and blood was collected and serum stored at -20°C for later determination of TNF- $\alpha$  concentrations. Colons were dissected, and the distal 8-cm rinsed with saline and scored for clinical macroscopic damage by an independent blinded observer, using a scale of 0-13 as set out below (Bobin-Dubigeon et al, 2001). A section of the affected colon was then collected, weighed, placed in a

80°C oven for 24 h, then reweighed and the wet-to-dry weight ratio determined as a measure of colon oedema (Rachmilewitz et al., 1989). A separate section of affected colon was also collected and homogenized with 1 mL phosphate buffered saline, sonicated for 20 seconds and centrifuged (14 000 x g, 10 min). The resulting supernatant was then either stored (-20°C) for later TNF- $\alpha$  determination, or used immediately in an assay for the determination of myeloperoxidase (MPO) levels. Briefly, the assay involved the addition of 20  $\mu$ L substrate (o-dianasidine, 2.85 mg/mL, Sigma, U.S.A.; and 0.85% hydrogen peroxidase) to a 1:40 dilution of supernatant in phosphate buffered saline. Absorbances were then read at 450 nm, 5 min after substrate addition for 24 h samples, and 15 min after addition for 8-day samples. Results for colon MPO content were converted to absorbance units/g tissue. Additional sections of colon were removed and stored in 10% formalin for histopathological analysis. Fixed colonic samples were embedded in paraffin wax, and sections were stained using a haematoxylin and eosin stain, photographed and examined by an independent observer in a blinded fashion. Tissue and serum TNF- $\alpha$  levels were determined from stored samples using an enzyme-linked immunoabsorbent assay as previously described (Arumugam et al, 2002), using undiluted samples. Results for colonic TNF- $\alpha$  content were then converted to pg TNF- $\alpha$ /g tissue.

Two time-frames were chosen for this study, an acute time frame (24 hours), and a chronic time frame (8-days). During the course of the study both body weight and food eaten were measured daily. In the 24 hour study, PMX53-, prednisolone- and combination-treated rats were treated daily, starting 2 days prior to instillation of TNBS (prevention). Rats treated with the TNF- $\alpha$  antibody, infliximab, were dosed intravenously on one occasion, 2 days prior to TNBS instillation. The following treatment groups were used in the 24 hour model:

- (a) PMX53 (10mg/kg, in olive oil, oral),

- (b) PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous),  
(c) C3a antagonist (30 mg/kg, bid, IP), prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous),  
5 (d) a combination of PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous), and prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous), and  
10 (e) infliximab (1mg/kg, in saline, intravenous).

In the 8-day study, drug-treated rats were treated both prior to colitis induction (2 days before; prevention) or following colitis induction (24 hours after; reversal). The following treatment groups were  
15 used in the 8-day study:

- (a) PMX53 (10mg/kg, in olive oil, oral, both pre- and post-induction),  
(b) PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous, pre-induction only),  
20 (c) prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous, both pre- and post-induction),  
(d) a combination of PMX53 (10mg/kg, in olive oil, oral) and prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous) (pre-induction only), and  
25 (e) infliximab (1mg/kg, in saline, intravenous, pre-induction only).

Colons were then macroscopically scored under blind conditions on a scale of 1-14, as follows:

30

<u>Ulceration</u>	<u>Diarrhea</u>	<u>Adhesions</u>
0- No damage	0- Absent	0- Absent
1- Focal hyperemia	1- Mild	1- Mild
2- Hyperemia and bowel thickening	2- Severe	2- Severe
3- Ulceration at 1 site		
4- Ulceration at 2 sites		

35



5- Ulceration >1cm  
6-10- Ulceration >2cm;  
increase score by 1 for each additional cm.

5                   The results for a number of parameters are  
summarized in Figures 1 and 2. All experimental results  
are expressed as means  $\pm$  standard error of the mean (SEM).  
Data analysis was performed using GraphPad Prism 3.0  
software (GraphPad Software, Inc. USA). Statistical  
10 comparisons were made between drug-treated groups and  
colitis control animals using a one-way analysis of  
variance followed by a Dunnett comparison post-test  
analysis. Statistical significance was assessed at  $P <$   
0.05.

15                   Mortality rate:       As shown in Figure 1, induction of  
colitis in drug-free rats resulted in a high mortality  
rate, 39%, beginning 2 days after TNBS administration. All  
drug treatments, except for prednisolone post-treatment,  
20 resulted in a decrease in mortality ( $n = 8-16$ ) compared  
with drug-free colitis control rats ( $n = 13$ ). No deaths  
were recorded in sham-operated animals ( $n = 4$ ). Rats  
treated with the C5a antagonist either prior to induction  
(10 mg/kg/day oral and 0.3 mg/kg/day SC) or 24 h after  
25 induction (10 mg/kg/day oral) had improved survival, with  
mortality rates of 11% or less. Rats pre-treated with the  
corticosteroid prednisolone (1 mg/kg/day SC) had a similar  
reduction in mortality rate, to 11%; however, post-  
treatment with prednisolone had no significant effect on  
30 reducing mortality. Infliximab pre-treatment (3 mg/kg IV)  
reduced the mortality only to 20%, which was much less  
effective than the C5a antagonist or prednisolone pre-  
treatment. There were no deaths among rats pre-treated  
with a combination of the C5a antagonist (10 mg/kg/day  
35 oral) and prednisolone (1 mg/kg/day SC).

Food Intake:   No significant improvements in food

consumption were seen after 24 h following drug treatment compared with drug-free colitis control rats. After 8 days all drug-treated rats except for prednisolone post-treated rats ate significantly more food than drug-free colitis control rats. As shown in Figure 2A, colitis induction resulted in a decrease in the consumption of food in all TNBS-administered rats compared to sham-operated rats after 24 h. Drug treatment did not significantly improve food intake over 24 h.

Over 8 days, as illustrated in Figure 2B, drug-free rats were still eating less food than sham-operated rats. Rats treated with the C5a antagonist either prior to induction (10 mg/kg/day oral and 0.3 mg/kg/day SC) or 24 h after induction (10 mg/kg/day oral) ate significantly more food than colitis control rats, and were consuming similar levels of feed to sham-operated rats at Day 8 ( $P < 0.05$ ). Rats pre-treated with prednisolone (1 mg/kg/day SC) also ate significantly more food than colitis control rats after 8 days ( $P < 0.05$ ); however, rats post-treated with this drug had no improvement in food consumption ( $P > 0.05$ ). Rats pre-treated with infliximab (3 mg/kg IV) also ate significantly more food than drug-free rats after 8 days, as did rats pre-treated with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC) ( $P < 0.05$ ).

Body weight: No significant reduction in weight loss was seen after 24 h following drug treatment compared with drug-free colitis control rats. After 8 days only rats treated with the C5a antagonist gained significant weight compared with drug-free colitis control rats. All rats receiving TNBS had considerable weight loss at 24 h post-induction compared to sham-operated rats, as shown in Figure 3A. Drug treatment did not significantly affect this weight loss over 24 h.

Over the 8-day study period, as shown in Figure 3B, drug-free rats continued to lose weight with the mean

weight loss at Day 8 being  $-21 \pm 8$  g ( $n = 8$ ). Rats treated with the C5a antagonist either prior to colitis induction (10 mg/kg/day oral and 0.3 mg/kg/day SC) or 24 h after induction (10 mg/kg/day oral) gained substantial weight over the 8 days, with significantly higher weights compared to colitis control rats ( $P < 0.05$ ). In contrast, rats either pre-treated or post-treated with prednisolone (1 mg/kg/day SC), or rats pre-treated with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC), lost weight over the 8 days, with no significant difference compared to colitis control rats on Day 8 ( $P > 0.05$ ). Rats pre-treated with infliximab (3 mg/kg IV) had only a small weight gain at Day 8; however, these levels were not significantly higher than colitis control rats ( $P > 0.05$ ).

Macroscopic Score: Colons were examined macroscopically for signs of haemorrhage and ulceration by an independent observer, in a blinded fashion, using a previously established scoring system (Bobin-Dubigeon et al, 2001). After 24 h, all drug treatments except for infliximab, resulted in a significant improvement in macroscopic scores compared with drug-free colitis control rats. After 8 days, all drug treatments except for prednisolone post-treatment resulted in a significant improvement in macroscopic scores compared with drug-free colitis control rats. Sham-operated animals displayed no appreciable macroscopic damage.

As shown in Figure 4A, after 24 h colons from all TNBS-treated rats were found to have extensive haemorrhage, oedema and early ulcer formation. No adhesions were observed, and diarrhoea was minimal. Drug-free rats had a mean macroscopic score of  $7.2 \pm 0.5$  ( $n = 12$ ) at 24 h. Pre-treatment of rats by any drug in the 24 h study resulted in reductions in macroscopic scores, with a significant improvement seen in all treatment groups except for infliximab ( $P < 0.05$ ). As shown in Figure 4B,

after 8 days colons from TNBS- treated rats had considerably greater damage than at 24 h, indicating that pathology was progressive. Although the colons had less haemorrhage, ulcer formation was extensive and adhesions and diarrhoea were common. Colitis control rats had a mean macroscopic score of  $11.5 \pm 0.8$  (n = 12) at Day 8. Rats treated with the C5a antagonist either prior to induction (10 mg/kg/day oral and 0.3 mg/kg/day SC) or 24 h after induction (10 mg/kg/day oral), had significantly improved macroscopic scores compared to colitis control rats, as did prednisolone pre-treated rats (1 mg/kg/day, SC) ( $P < 0.05$ ). Rats pre-treated with infliximab (3 mg/kg IV) also had significantly decreased scores on Day 8 ( $P < 0.05$ ). Rats post-treated with prednisolone (1 mg/kg/day, SC) did not have any improvement in macroscopic scores on Day 8 ( $P > 0.05$ ).

Colon MPO and Oedema: After 24 h, only rats treated with either the C5a antagonist or a combination of the C5a antagonist and prednisolone, had a significant improvement in colon MPO levels compared with drug-free colitis control rats. After 8 days, all drug treatments except for prednisolone post-treatment resulted in a significant improvement in colon MPO levels compared with drug-free colitis control rats. As shown in Figure 5A, levels of MPO in the colons of drug-free rats were substantially higher at 24 h than in sham-operated rats. Levels of this enzyme were reduced in all drug-treated rats after 24 h, with a significant improvement in rats either pre-treated with the C5a antagonist (10 mg/kg/day oral or 0.3 mg/kg/day SC) or with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC) ( $P < 0.05$ ).

As shown in Figure 5B, after 8 days the levels of colonic MPO in TNBS-administered rats were further reduced compared with 24 h. In rats treated with the C5a antagonist either prior to induction (10 mg/kg/day oral

and 0.3 mg/kg/day SC) or 24 h after induction (10 mg/kg/day oral), or in rats pre-treated with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC), the levels of colonic MPO were reduced to similar levels to those of the sham-operated animals after 8 days ( $P < 0.05$ ). Rats pre-treated with either prednisolone (1 mg/kg/day SC) or infliximab (3 mg/kg IV) also had significantly reduced colonic MPO levels after 8 days, although the extent of improvement was not as great as in rats treated with the C5a antagonist ( $P < 0.05$ ). Rats post-treated with prednisolone (1 mg/kg/day SC) did not show any improvement in colonic MPO levels ( $P > 0.05$ ).

Colonic oedema: Colon edema, determined by wet-to-dry weight ratios, was measured at either 24 h or 8 days. The results are shown in Figure 6. After both 24 h and 8 days, only rats treated with either the C5a antagonist, or a combination of the C5a antagonist and prednisolone, had a significant improvement in colon wet-to-dry weight ratios, compared with drug-free colitis control rats. As shown in Figure 6A, wet-to-dry weight ratios of colons from TNBS-administered rats were considerably increased compared to sham-operated rats after 24 h, indicating oedema. At this time point, only rats which had been pre-treated with the C5a antagonist (10 mg/kg/day oral or 0.3 mg/kg/day SC) or with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC), had a significant reduction in wet-to-dry weight ratios compared to colitis control rats ( $P < 0.05$ ). The C3a antagonist did not have a significant effect.

As shown in Figure 6B, after 8 days the level of colonic oedema in TNBS-administered rats was still considerably higher than for sham-operated rats, although lower than that seen at 24 h. There were significant reductions in wet-to-dry weight ratios in rats treated with the C5a antagonist, either prior to induction (10 mg/kg/day oral and 0.3 mg/kg/day SC) or 24 h after

induction (10 mg/kg/day oral), as well as in rats pre-treated with a combination of the C5a antagonist (10 mg/kg/day oral) and prednisolone (1 mg/kg/day SC) in this 8 day time frame ( $P < 0.05$ ). Post-treatment with prednisolone (1 mg/kg/day SC) had no effect on reducing edema, whilst prednisolone (1 mg/kg/day SC) and infliximab (3 mg/kg IV) pre-treated rats had no significant reductions compared to colitis control rats ( $P > 0.05$ ).

10 Serum and Tissue TNF- $\alpha$  Levels: TNF- $\alpha$  levels were then measured after 8 days in either serum or colonic tissue. The results are illustrated in Figure 7. Serum levels of TNF- $\alpha$  were increased in drug-free colitis control animals, and all drug treatments significantly reduced these

15 levels. All drug treatments, except for prednisolone post-treatment, significantly improved colonic TNF- $\alpha$  levels compared with drug-free colitis control rats. Levels of serum TNF- $\alpha$  and colonic tissue TNF- $\alpha$  were not increased in TNBS-treated rats compared to sham-operated

20 rats after 24 h. As shown in Figure 7A, after 8 days serum TNF- $\alpha$  levels in colitis control rats were increased compared to sham-operated rats. All drug-treated rats had similar serum TNF- $\alpha$  levels to sham-operated rats, and these levels were significantly reduced compared to

25 colitis control levels ( $P < 0.05$ ). Colon tissue homogenate TNF- $\alpha$  levels; shown in Figure 7B, were also increased in drug-free rats compared to sham-operated animals. All drug-treated rats had significantly lower tissue levels of this cytokine, compared to colitis

30 control rats ( $P < 0.05$ ), except for prednisolone post-treated rats (1 mg/kg/day SC), which had no significant reduction ( $P > 0.05$ ).

Histopathology: Stained sections of colon were

35 examined for signs of pathology by an independent observer in a blinded fashion. At 24 h after TNBS administration, sections of colons showed signs of acute inflammation. In

mild cases there was oedema of the submucosal layers of the bowel and infiltration, predominantly by neutrophils, into the mucosa and submucosa. In more severe cases there was necrosis of the colonic mucosal cells, and more severe oedema and inflammatory cell infiltration. No observable improvements were seen in sections of colons from drug-treated rats compared to drug-free colitis control rats in this acute time frame.

At 8 days after TNBS challenge there was evidence of healing, with affected mucosa being replaced by fibrous tissue. There was PMN infiltration of affected tissues. Where discrete ulcers were identified, the bordering mucosa appeared normal, although there was generalised submucosal inflammation, consisting of inflammatory cells and mild oedema. Colon sections from drug-free colitis control rats showed the most severe pathology after 8-days (Figure 8B). In comparison, sections of colon from sham-operated rats showed minimal signs of damage (Figure 8A). After 8 days sections of colons from all rats which had been pre-treated with either the C5a antagonist, prednisolone, infliximab or a C5a antagonist/ prednisolone combination showed an improvement in pathology compared to drug-free rats. Sections of colons from rats which had been post-treated with the C5a antagonist (10 mg/kg/day PO) also showed an improvement in disease pathology after 8 days, indicated by less ulcer formation, oedema, inflammatory cell infiltration, no perforations and intact mucosal layers compared with sections from drug-free rats (Figure 8C). In contrast, sections of colons from rats which had been post-treated with prednisolone (1 mg/kg/day SC) showed no improvement in disease pathology compared with drug-free rats (Figure 8D).

In the 24-hour study, PMX53-treated rats had significantly lower colon macroscopic scores and higher body weights and food intake. The level of colon edema, neutrophil accumulation and colon TNF- $\alpha$  levels were also significantly reduced. In comparison to prednisolone and

infliximab, which are currently used in therapy for IBD, PMX53-treated rats displayed a greater inhibition of the parameters measured. These results show that blockade of the inflammatory protein C5a by PMX53 significantly  
5 improves disease pathology in the acute 24-hour model of IBD, to an extent at least as great as that achieved by prednisolone or infliximab.

In the 8-day study, PMX53 pre-treated rats as well as post-treated rats lost significantly less body  
10 weight and ate significantly more food compared to colitis control animals. These rats also had significantly lower colon macroscopic scores, colon edema, neutrophil accumulation and colon TNF- $\alpha$  levels. Most importantly, PMX53-treated rats also had reduced mortality compared to  
15 colitis control rats. In comparison, only prednisolone pre-treated rats displayed any improvement in the disease parameters measured. Infliximab-treated rats also had significantly reduced disease parameters, however to a lesser extent than those observed in PMX53-treated rats.

Histologically, colonic sections of rats treated with PMX53 displayed a decrease in inflammatory cell accumulation and haemorrhage formation, and this is shown in Figure 3. These results surprisingly show that  
20 blockade of the inflammatory protein C5a by PMX53 both prevents and reverses disease pathology in a rat model of chronic colitis. This effect of reversal of pathology was  
25 not seen in rats treated with prednisolone.

In summary, these studies demonstrate for the first time that an inhibitor of the complement system has  
30 beneficial effects in an established model of IBD. The improvements seen with PMX53 were greater than those seen with prednisolone or infliximab. This indicates that PMX53 will be useful in the clinical treatment of IBD.

35 Example 2: Assessment of clinical efficacy

The clinical efficacy in humans of compounds found to be effective in animal models may be determined



using standard clinical trial methods.

For example, in a randomized, placebo-controlled, phase II trial in the treatment of patients with mild-to-moderate Crohn disease will typically employ at least two dose levels of the test compound. A decrease of greater than 70 points on the Crohn's Disease Activity Index (CDAI), a standardized tool designed to measure disease activity specifically, is a primary endpoint for indication of efficacy. Disease remission is a secondary endpoint. A treatment effect advantage is established if significantly more recipients of the test compound than placebo recipients are found to have CDAI scores consistent with symptom resolution during an acute exacerbation of the inflammatory bowel disease.

For ulcerative colitis, the effects of treatment with the test compound are assessed in patients with symptoms of active ulcerative colitis who have either not previously been treated, or who are also receiving standard medical treatment. The endpoints are induction of complete remission or significant improvement in signs and symptoms of ulcerative colitis, as reflected in changes in a colitis activity index (CAI) score. The CAI score assesses stool frequency, rectal bleeding, endoscopic appearance of the colon, and includes a physician's global assessment.

For each condition the adverse effect and tolerability profiles of the test compound are also monitored.

### 30 DISCUSSION

Cyclic peptides have several important advantages over acyclic peptides as drug candidates (Fairlie et al 1995, Fairlie et al, 1998, Tyndall and Fairlie, 2001). The cyclic compounds described in this specification are stable to proteolytic degradation for at least several hours at 37°C in human blood or plasma, in human or rat

gastric juices, or in the presence of digestive enzymes such as pepsin, trypsin and chymotrypsin. In contrast, short linear peptides composed of L-amino acids are rapidly degraded to their component amino acids within a few minutes under these conditions. A second advantage lies in the constrained single conformations adopted by the cyclic and non-peptidic molecules, in contrast to acyclic or linear peptides, which are flexible enough to adopt multiple structures in solution other than the one required for receptor-binding. Thirdly, cyclic compounds such as those described in this invention are usually more lipid-soluble and more pharmacologically bioavailable as drugs than acyclic peptides, which can rarely be administered orally. Fourthly, the plasma half-lives of cyclic molecules are usually longer than those of peptides.

Models of IBD typically involve the intra-colonic administration of an inflammatory or haptensising agent. The TNBS-induced colitis model used herein is a simple, reliable model which has been widely used to examine the efficacy of various candidate drugs for treatment of IBD.

We found that 24 h after TNBS administration, rat colons displayed large areas of haemorrhage, oedema and inflammatory cell infiltration. However, in this acute model we found it difficult to detect any significant therapeutic effects for drugs compared to drug-free, colitis-bearing rats. In contrast, 8 days after colitis induction the colons of rats showed considerably greater damage than seen at 24 h, and it became easier to compare the effects of different drugs. The increased damage observed at 8 days confirms that the pathology continues to develop, even after the initiating agent has been metabolized. In this chronic model a substantial proportion of the rats died, and elevated levels of TNF- $\alpha$  in serum and colonic tissue were observed in the surviving rats. The 8 day experiment also allowed the inclusion of post-induction drug treatments, to determine whether drugs

could be used to treat and perhaps reverse the developing disease.

We used both the 24 h and 8 day time frames to compare the efficacy of two newly- developed antagonists of the C3a and C5a receptor with that of the steroid prednisolone and the TNF- $\alpha$  inhibitor infliximab. All the drugs tested were effective at reducing some, or all, of the parameters measured. However, the C5a antagonist was found to be superior to the other drugs.

Corticosteroids, such as prednisolone, are commonly prescribed therapies in the treatment of IBD. They are powerful drugs which act at the genetic level, resulting in the downregulation of various pro-inflammatory mediators, such as cytokines, as well as immune pathways. Because of their non-specific actions corticosteroids have numerous serious side effects, and must be used with caution in patients, particularly for chronic treatment. In our study, prednisolone pre-treatment was effective at reducing the majority of parameters measured, as expected. However, prednisolone-treated rats lost considerable body weight. This was attributed to catabolism, a major side effect with steroid therapy. Although steroids are widely used in colitis therapy, it is of interest that rats treated with steroid 24 h after the induction of colitis showed no appreciable therapeutic effects. Because of the time it takes for steroids to become effective, this may have been too late for any appreciable effect of prednisolone on disease pathology. Alternatively, the lack of effect may have resulted from the tendency of steroid treatments to inhibit natural healing processes associated with disease pathology.

The proinflammatory cytokine TNF- $\alpha$  is thought to be a major contributor to the pathology of IBD. Recent advances in drug development have produced various antibodies which block the effects of this cytokine (Muller, 2002). One of these antibodies, infliximab, has

been reported to be effective in reducing IBD pathology, and is now currently available for the treatment of IBD in various countries. Such antibody-based therapies have delivery problems in the clinic, and are significantly  
5 more expensive than other drug therapies (Valle et al, 2001).

The efficacy of infliximab in an animal model of IBD has not to our knowledge been previously described. We therefore used this compound as a comparator drug for  
10 the C5a and C3a receptor antagonists. Although pre-treatment of rats with infliximab did reduce the severity of lesions, it was not as effective as the C5a antagonist or prednisolone. Infliximab was more effective in rats treated for 8 days than for rats in the 24 h study. Given  
15 that detectable increases in serum and colonic tissue TNF- $\alpha$  levels were only seen in the 8-day study, the effect of TNF- $\alpha$  inhibition by this compound would be expected to be minimal during the acute stages of the disease.

Abnormal complement activity is suspected to be  
20 important in the pathology of IBD, although there is limited evidence for this to date. In this study, we examined the efficacy of the C3a antagonist SB 290157. This compound has been recently reported to be an antagonist of the human C3a receptor, and to show activity  
25 in various *in vitro* and *in vivo* assays (Mollnes et al, 2002; Ames et al, 2001). We found that this antagonist was effective in reducing some of the parameters measured in the 24 h study. Most importantly, it reduced the intensity of macroscopic damage compared to colitis  
30 control rats. The dosage regime chosen in our study (30 mg/kg i.p. twice daily) was the same as used in previous *in vivo* studies, and this high dose appears to be necessary to obtain therapeutic effects with this compound (Ames et al, 2001). Because of the need to inject animals  
35 intraperitoneally twice daily, the drug was not evaluated in the 8-day model. Despite the inhibitory effects seen with SB 290157 after 24 h, further studies will need to be

performed over a longer time to identify the precise role of C3a in TNBS-induced colitis.

The cyclic peptide C5a antagonist PMX53 (AcF-[OPdChaWR]) was the most effective agent used in this study. We found that pre-treatment of rats with the C5a antagonist either orally or SC markedly reduced all the disease markers measured. Rats which were treated orally 24 h after colitis was established also had a significant reduction in all disease markers. This C5a antagonist has been shown to be specific for the C5a receptor in human, rat and dog (Woodruff et al, 2001), and to be specific for the C5a receptor over other related human receptors (Finch et al, 1999). In particular, PMX53 does not bind to the C3a receptor (Finch et al, 1999), and has no effect on the formation of the membrane attack complex (Arumugam et al, 2003). The increased efficacy seen with the C5a antagonist over the C3a antagonist, at least in the 24 h study, is consistent with the higher potency and the greater pro-inflammatory activity of C5a compared to C3a *in vivo*.

The greater efficacy of the C5a antagonist compared to infliximab may be explained by the central role of C5a in the inflammatory cascade. It is known that C5a induces the release of not only TNF- $\alpha$ , but also a host of other inflammatory cytokines and various mediators which are reportedly also involved in IBD (Haynes et al, 2002; Mollnes et al, 2002). This C5a antagonist is also known to prevent formation of a number of these mediators (Finch et al, 1999; Haynes et al, 2002), which probably explains its superior efficacy to infliximab, which solely inhibits TNF- $\alpha$ . We have now shown that an antagonist of human C5a receptors is also a potent and effective treatment for IBD in a rat model.

When rats were pretreated with a combination of the C5a antagonist and prednisolone, no greater reduction in disease markers was observed than with the C5a antagonist alone. This suggests a common downstream

regulation of inflammatory mediators.

In summary, this study demonstrates a significant role for the complement components C3a, and especially C5a, in the pathogenesis of TNBS-induced colitis in rats.

5 Complement may therefore have a pivotal role in the development of human IBD. This should encourage the development of anti-complement-based therapies. The potent therapeutic effects of the orally active human C5a antagonist AcF-[OPdChaWR], and its greater efficacy  
10 compared to prednisolone and infliximab, support a role for the use of C5a antagonists in the treatment of IBD in humans.

15 It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing  
20 from the scope of the inventive concept disclosed in this specification.

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FIGURE 1

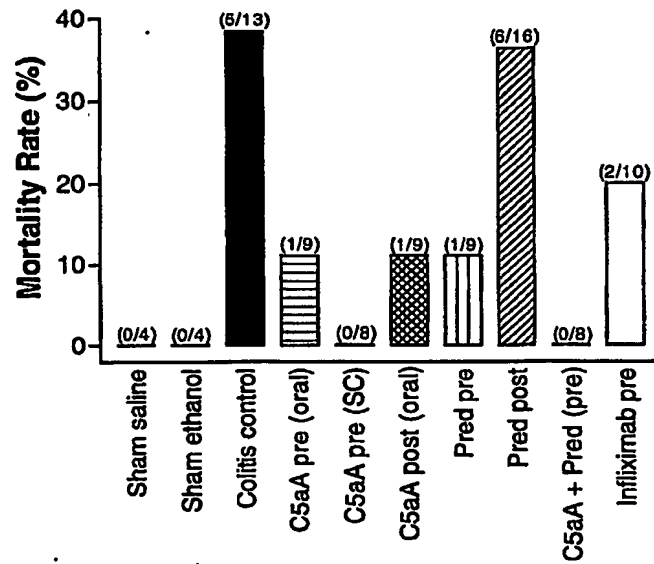




FIGURE 2

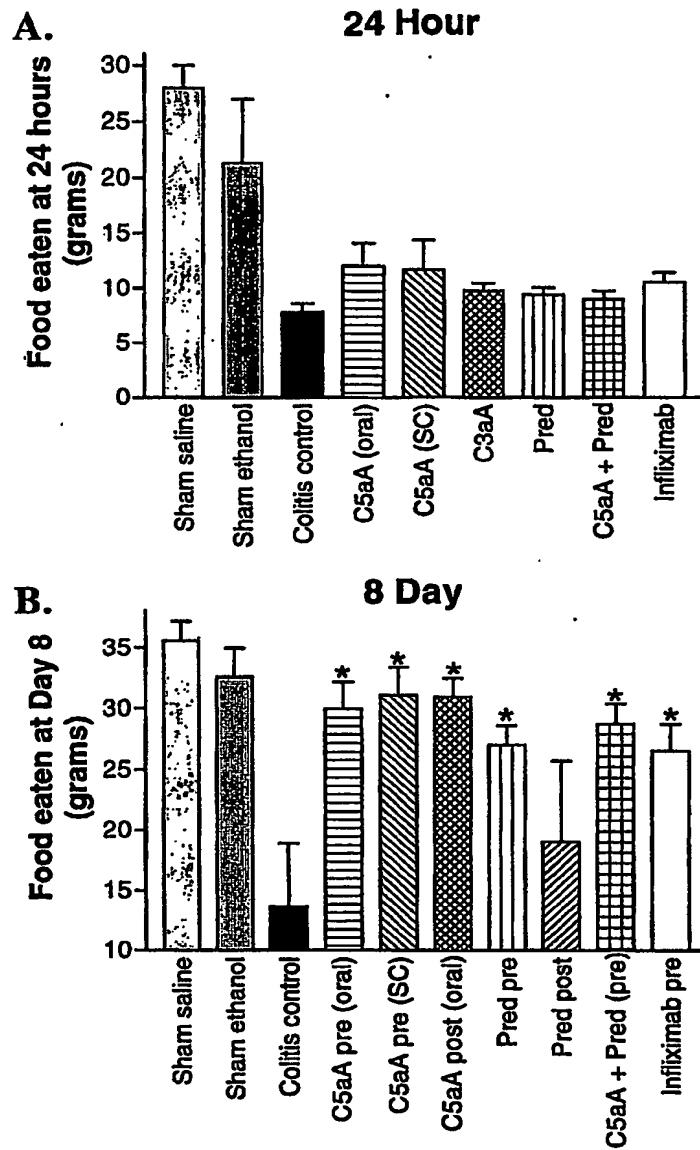


FIGURE 3

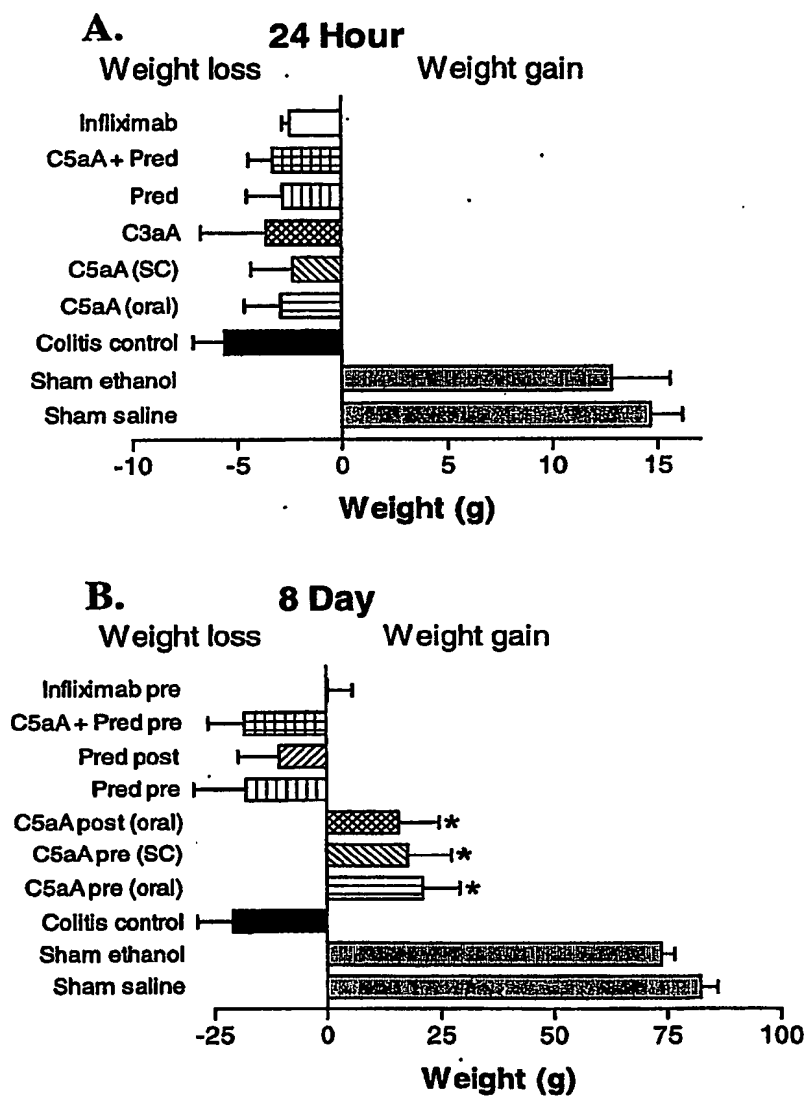
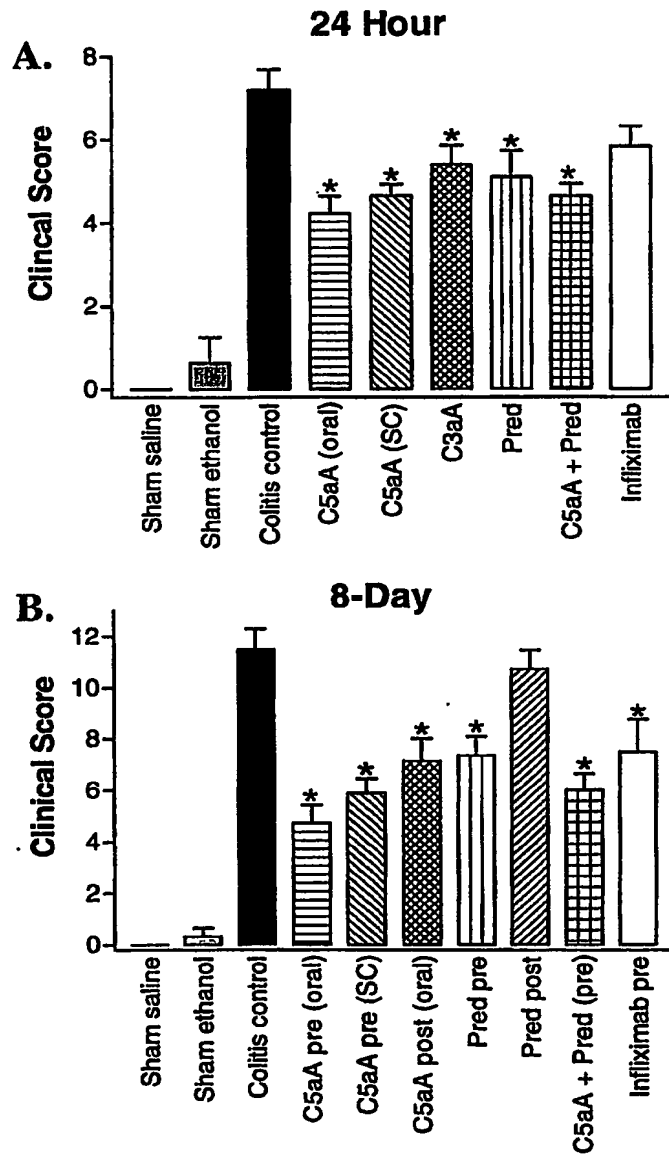


FIGURE 4



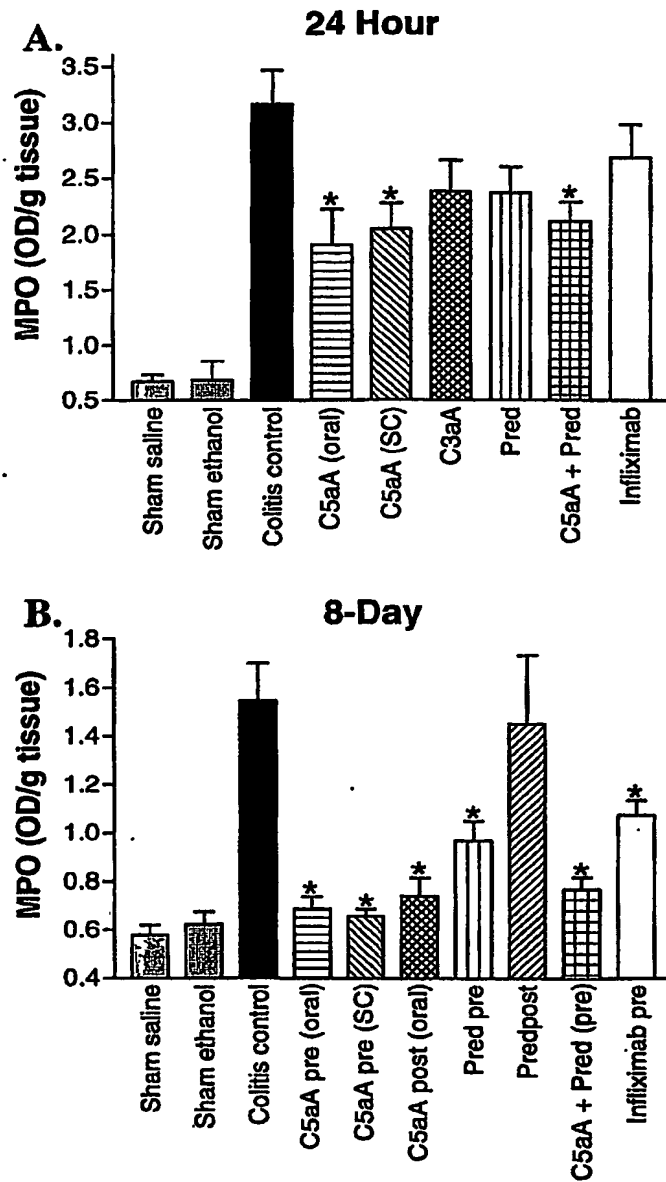


FIGURE 5

FIGURE 6

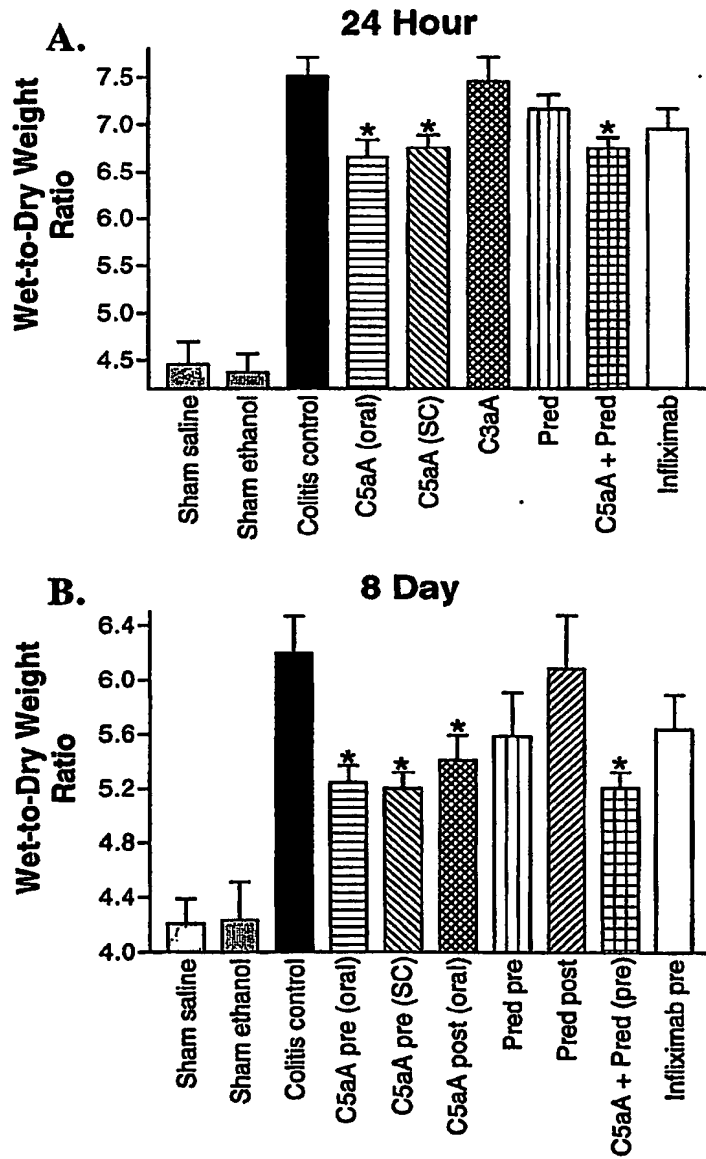


FIGURE 7

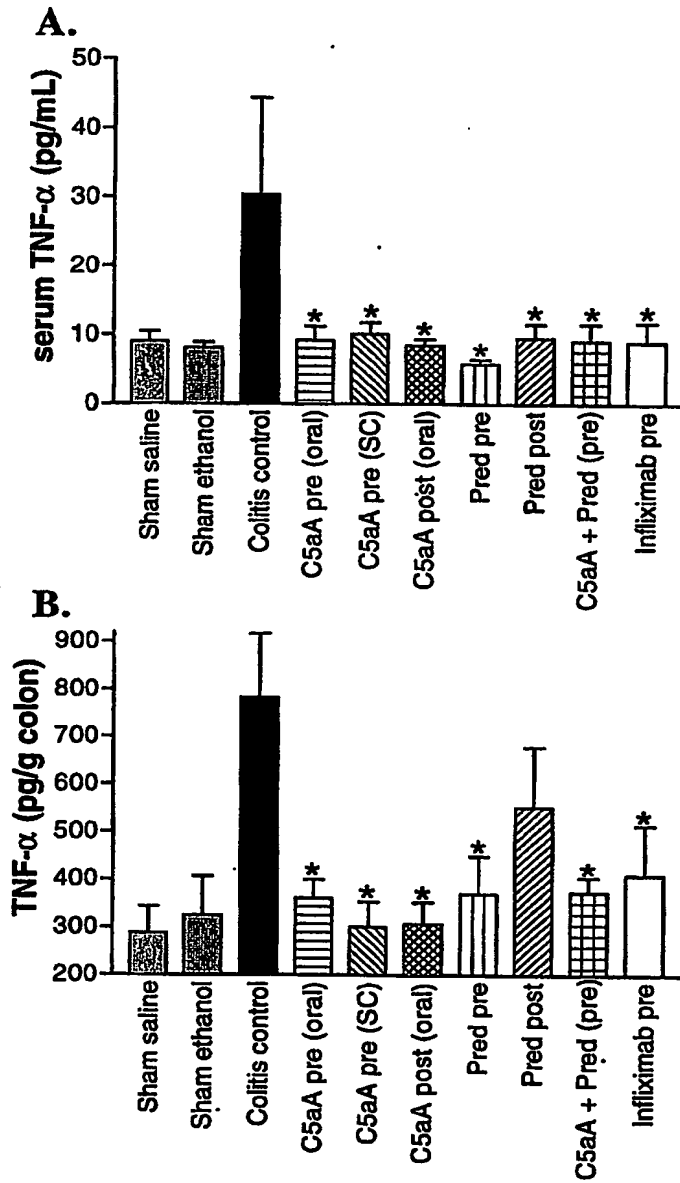


FIGURE 8

